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Screening of Reduced Risk Compounds for Fungicidal and Herbicidal Activity

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This is part of an on-going research project covered under the Cooperative Research and Development Agreement #58-3K95-1-0853. A select group of compounds have been screened using laboratory and greenhouse bioassays for their effects on the fungus *Fusarium oxysporum* f. sp. *lycospersici*, and the weeds purple nutsedge (*Cyperus rotundus*), smooth pigweed (*Amaranthus hybridus*), and barnyard grass (*Echinocloa crus-galli*). Each compound screened falls under all of the three following categories: 1) reduced risk, 2) biodegradable, and 3) non-ozone depleting.

The evaluation of fungicidal activity was performed using a modified colorimetric assay (Mace et al., 1990), referred to as the *Fusarium* tetrazolium assay. Each candidate compound was tested using a suspension of *F. oxysporum* f. sp. *lycopersici* (isolate FP-7) spores harvested from 10-day old petri plates using a 0.05% stock solution of Triton X-100 (referred to as FP). Harvested spores were suspended in Czapek-Dox Broth (CDB), prepared according to label instructions, at ratio of 1:5 CDB:FT. The suspension was then standardized using a Beckman spectrophotometer (430nm), which has been calibrated using a CDB blank. Compounds were then added to aliquots of the suspension to achieve active ingredient concentrations ranging from 0 ppm (untreated control) to 2000 ppm. Suspensions were incubated for 24 hr at 28 C. A 10% stock solution of 3-(4,5-dimethylthiazol-2 yl)-2,5-diphenyltetrazolium bromide (MTT) was then added to each aliquot. The suspensions were allowed to incubate for 4 hr, were then pelleted, drained, rinsed, and treated with 95% ethanol. The color change, which correlates with spore viability, is assessed using the Beckman spectrophotometer (570 nm), calibrated with 95% ethanol. Benlate (benomyl) was used as a standard control. Each treatment was replicated six times and the assay for each compound was repeated. Regression analysis was performed using Sigma Plot 2000.

Each candidate compound was tested on seeds of smooth pigweed and barnyard grass, and on tubers of purple nutsedge by exposing seeds or tubers to a specific concentration of the test compound. Seeds were surface sterilized with 10% bleach solution and 20 seeds placed on sterile filter paper (Whatman #5) in a 10-cm petri plate for each of six replicates for each weed species tested. Twenty ml of compound of concentrations ranging from 0 ppm (sterile water control) to 2000 ppm was added to the petri dishes. Plates were stored at 30 C in a dark incubator. Seed germination was monitored at 24 hr intervals for 30 days. Petri plate bioassays were repeated twice for each compound. Field collected purple nutsedge tubers were placed in 500g of field soil with soil moisture adjusted to 5%. Five nutsedge tubers were planted in each 10-cm pot at a depth of 2.5 cm. Six replicate pots of each concentration, ranging from 0 ppm (water control) to 2000 ppm, were treated using 100 ml as a soil drench. Pots were tarped with co-extruded black-on-white polyethylene mulch for 7 days. After tarps were removed, the number of emerged nutsedge shoots was recorded on a weekly basis for 60 days.

Two compounds, AJMC-330 and AJMC-334, have shown particularly good results in these screens. Results from the *Fusarium* tetrazolium assay appear in Figures 1 A&B. The assay is based on a color change produced with a vital stain on living tissue, i.e. the lower the absorbance, the fewer viable cells. In this test, AJMC-334 was comparable to the Benlate check, with efficacy at the 100-500 ppm range. AJMC-330 was effective at reducing cell viability to levels achieved with Benlate (100 ppm) at concentrations between 500 and 1000 ppm.